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Experimental referred pain extends toward previously injured location

An explorative study

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EXPERIMENTAL REFERRED PAIN EXTENDS TOWARDS
PREVIOUSLY INJURED LOCATION – AN EXPLORATIVE STUDY

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Highlights

- It is unclear if a recovered pain condition leaves traces of higher sensitivity of pain mechanisms
- Pain is more frequently referred to the previous area of nociceptive activity
- The pain area in the previously painful area is enlarged compared with controls
- The ability to dampen pain via endogenous inhibition seems improved after recovery from pain
- The findings shed a light on the mechanisms involved in recovery from musculoskeletal pain

EXPERIMENTAL REFERRED PAIN EXTENDS TOWARDS PREVIOUSLY INJURED LOCATION – AN EXPLORATIVE STUDY

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Running head: *Referred pain, musculoskeletal injury, neuroplasticity*

Disclosures

SAB is the co-developer of the software application Navigate Pain v1.0 (Aalborg University) used to collect the pain drawings. SAB has company holdings in Aglance Solutions ApS which licenses Navigate Pain. Nocitech is partly owned by Aalborg University. This study was not financially supported from any third party stakeholders. The remaining authors report no conflicts of interest. Center for Neuroplasticity and Pain (CNAP) is supported by the Danish National Research Foundation (DNRF121).

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ABSTRACT

Facilitated pain mechanisms have been demonstrated in musculoskeletal pain but it is unclear whether a recent painful injury leaves the pain system sensitized. Pain characteristics were assessed in individuals who recently recovered from ankle pain (recovered pain group; $n=25$) and sex-matched controls ($n=25$) in response to tonic-pressure pain and saline-induced pain applied at the shin muscle. Pain intensity and pain referral patterns were recorded bilaterally after the painful muscle stimulus. Pressure pain thresholds (PPTs) were measured at the lower legs and shoulder. Cuff pressure algometry on the lower leg was used to assess pain detection threshold (PDT), pressure evoking 6-cm pain score on a 10-cm visual analogue scale (PVAS6), pain tolerance (PTT), temporal summation of pain (TSP), and conditioned pain modulation (CPM). Compared with controls, saline-induced and pressure-induced pain in the shin muscle were more frequently felt as referred pain in the previously painful ankle ($P<0.05$) and the pain area within the previously affected ankle was larger following saline-induced pain ($P<0.05$). In the recovered pain group, CPM responses and the cuff pressure needed to reach PVAS6 was higher in the previously painful leg compared with the contralateral leg ($P<0.05$). No group differences were found in PPT, PDT, PTT, and TSP.

Perspectives

These explorative findings demonstrate that pain mechanisms responsible for pain location may be reorganized and continue to be facilitated despite recovery. A large prospective study is needed to clarify the time profile and functional relevance of such prolonged facilitation in the pain system for e.g. understanding recurring pain conditions.

INTRODUCTION

Musculoskeletal pain affects a vast majority of the population at some stage during the lifespan⁴. After an initial painful musculoskeletal event, such as whiplash or an acute back sprain, recurrent episodes of pain separated by pain-free periods are common^{11,18,31}. Although, the mechanisms for pain recurrence are unclear unresolved tissue pathology is not considered a driving factor²⁴.

Several studies have demonstrated that facilitated central pain mechanisms are associated with persistent and widespread pain in a number of musculoskeletal patient populations¹⁵. The central processing of on-going nociceptive stimuli continues until it gradually subsides following the healing of trauma-related tissue damage. Ongoing nociceptive stimuli can drive neuroplasticity and contribute to facilitated central pain mechanisms²⁷. In chronic pain conditions maladaptive neuroplasticity may manifest as increased pain sensitivity^{10,15}, facilitated temporal summation of pain⁴⁵, reduced efficacy of descending pain inhibition assessed by conditioning pain modulation (CPM)⁴⁴, and expanded pain referral¹⁵. Furthermore, individuals recovering from acute low back pain may present with impaired CPM³³ and facilitated temporal summation⁴³. It is however unclear whether such an enhancement is still present in individuals who have recovered from painful injury.

In healthy subjects, weekly intramuscular injections of hypertonic saline inducing pain with short duration can lead to progressively larger areas of referred pain³⁹. The repetitive nature of the nociceptive insult may underlie the progressive expansions in the referred pain. In general, the mechanism for location of referred pain is not fully understood. Saline-induced pain in the tibialis anterior or brachioradialis muscles frequently but not always gives referred pain to the ankle and wrist area, respectively¹⁴. Interestingly, an experimental nociceptive stimulus to the maxillary sinus resulted in a pain experience at the site of a recent dental procedure²¹. These experimental findings may indicate that individuals with a recent painful injury may be more susceptible to a subsequent painful event evoking referred pain to the area originally traumatized.

This study was designed to assess the referred pain response to experimental pain as well as the pain sensitivity, temporal pain summation, and CPM in individuals recently recovered from ankle pain, as compared to controls. It was hypothesized that i) pain referral pattern induced from the tibialis

anterior muscle towards the ankle region is larger and occurs more frequently in individuals who recently recovered from a pain condition in the ankle, and ii) in these individuals residual effects of changed central pain mechanisms are reflected as increased pain sensitivity, a facilitated temporal pain summation and reduced CPM. The goal of this explorative study was to assess residual effects in central pain mechanisms that may contribute to recurrent pain and form basis for a future large-scale study.

METHODS

Subjects

Asymptomatic male and female participants were recruited from a university population and local sports clubs and divided into two groups: A group with prior history of soft-tissue injury or idiopathic pain from the ankle region, within 3 months from the data collection (recovered pain group) as well as a control group consisting of sex and age matched individuals.

Recovered pain group

Participants were recruited in this group if they had a recent history of on-going ankle pain with or without an injury were included, pending a return to normal daily function and without spontaneous or on-going pain in the ankle or lower limb (i.e. completely asymptomatic). No effort was made to differentiate between pain conditions related to an injury (e.g. an ankle sprain) or pain that was brought on spontaneously with or without a previous history of an injury to the area. This was done to allow their recent pain condition to be related with a previous injury (>12 weeks) that had recovered but was aggravated by e.g. strenuous physical activity which they had experienced within the 12 weeks prior to recruitment. The rationale behind this was the high recurrence rates of ankle and foot injuries after the first episode⁶ and therefore the small chances of finding adults with only a single, recent lifetime episode of pain or injury to the foot or ankle. This was therefore noted at inclusion (see table 1) but not used as an exclusion criteria. Participants in the recovered pain group had been pain-free for a minimum of 2 weeks prior to participation after recovering from an injury or a pain condition that limited normal function within the previous 3 months.

Control group

The volunteers in the control group were likewise pain-free at the time of data collection. Due to the fact that injuries to the foot and ankle are frequent, especially in childhood and adolescence⁷, only persons with no previous history of substantial ongoing pain or injury to the lower limb/ankle regions were included. A substantial ongoing pain or injury was defined as fracture to the foot or ankle or a surgical procedure aimed at rectifying a musculoskeletal injury in the area. Subjects with a recent (within 12 weeks) history of pain or minor injury to the ankle/foot were not included in the control group.

For both groups, a current or recent (within 3 months) significant pain condition to other body parts/regions was an exclusion criterion. All participants were naïve to the experimental procedure at inclusion in the sense that they had not participated in a similar experimental pain study previously. A total of 55 healthy participants (26 females) with a mean age of 24 years (range 18 – 35 years), a mean weight of 74 kg (range 49 – 110 kg), and an average height of 176 cm (range 156 – 200 cm) were recruited for the study.

Prior to inclusion, participants were screened by the principal investigator (TSP) who is an experienced physiotherapist specialized within musculoskeletal pain. All participants were healthy and completely pain-free at inclusion. Participants who were pregnant or reporting any systemic diseases were excluded. For both groups, any history of surgery of the lower limbs and use of any form of medication at present or on-going was noted at inclusion but not considered a direct exclusion criteria. In case any of the study participants did not tolerate the experimental procedures, they were excluded from the study and all collected data from them was removed from the final dataset.

Participants received a written and oral description of the study prior to giving their informed consent. The study was conducted in accordance with the Helsinki Declaration and approved by the regional Ethics Committee (N-20120004).

Experimental protocol

A flow-chart of the study protocol is shown in figure 1. The study was a single blinded (assessor was blind to group allocation), cross-sectional group comparison conducted in one 90-minute session where participants rested in a reclined sitting position. First, the pain sensitivity was assessed by pressure pain

thresholds (PPT) at three bilateral body sites. Secondly, the distribution of experimentally induced pain referral was assessed by two different methods applied to the tibialis anterior muscle (initially by tonic pressure pain stimulation, then by hypertonic saline injection, and subsequently by tonic pressure pain stimulation). In a balanced randomized order, experimental pain referral was induced first in the uninjured leg or the previously painful leg (test leg) for the recovered pain group. The same randomization approach was used for the dominant leg (defined as test leg) and non-dominant leg in the control group. PPTs were reassessed during saline-induced pain and following a 5 min pain-free period. Subsequently, cuff pressure pain sensitivity and temporal summation of pain was assessed using cuff algometry on both legs starting with the test leg. Finally, cuff algometry was used to assess the effectiveness of endogenous pain inhibition by using a conditioning pain modulation paradigm with the conditioning stimulation on the test leg and the cuff test stimulus on the contralateral leg. The hypotheses of the study were not introduced to any of the subjects prior to their participation.

Pressure pain sensitivity

Pressure-pain thresholds (PPT) were assessed bilaterally and marked for multiple assessments: (1) The deltoideus muscle, over the bulky medial part of the muscle, midway between acromion and the deltoid tuberosity. (2) The tibialis anterior (TA) muscle, two fingerbreadths medial and two fingerbreadths inferior to the fibular head. (3) The talocrural joint, at the mid-point between the medial and lateral malleolus immediately lateral to the tendon of the tibialis anterior muscle (Fig. 2). A handheld pressure algometer (*Algometer*[®], *Somedic, Sweden*) with a 1 cm² probe (covered by a disposable latex sheath) was used to apply increasing pressure with a ramp of 30 kPa/s. The PPT was defined as the moment the pressure became painful as indicated by the participants pressing of a button to end the test. At baseline, the three individual PPTs were acquired on each site with a minimum 30 s between assessments. The subsequent assessments were based on two PPT assessments for each time point. The average of the PPTs acquired at each site for each time point was used for statistical analysis and for calculating the stimulation intensity used for tonic pressure-induced pain. PPTs were normalized with the baseline values ('during pain' and 'post pain' divided by 'baseline' values).

Tonic pressure-induced pain referral

The tonic pressure pain stimulation intensity was defined as 120% of the baseline PPT assessments and applied to the TA muscle site for 60 s using a computer-controlled pressure algometer (*Aalborg University*). The participant could ‘stop’ the supra-pain threshold pressure pain stimulation by pressing a handheld button. During the stimulation, the leg was stabilized using a vacuum pillow (*AB GERMA, Kristianstad, Sweden*).

Saline-induced pain referral

Sterile hypertonic saline (1 ml, 5.8%) was injected over approximately 10 s into the belly of the TA muscle (same site as used for pressure algometry). Prior to injection, the skin was cleaned using standardized disinfection protocols. Injections were performed using a 2 ml plastic syringe with a disposable needle (27G). Recording of the pain intensity was initiated immediately after the injection using a 10-cm electronic visual analogue scale (VAS) with an external handheld slider to adjust the scale. The VAS was anchored with ‘no pain’ and ‘maximum pain’, 0 cm and 10 cm, respectively. The VAS signal was recorded until the perception of pain subsided (sampling frequency of 25 Hz). The peak pain (VAS-peak) and area under VAS-time curve (VAS-area) were extracted and used for data analysis. The pain duration was defined as the time difference between the first and the last time the VAS score was greater than zero. In case VAS scores did not change from baseline, the pain duration was set to zero.

Assessment of saline and tonic pressure-induced pain area

After the saline or tonic pressure-induced pain subsided, participants were asked to draw the area of pain and extent on an electronic three-dimensional body chart of the lower legs (anterior view) on a 10.1” tablet (*Samsung Galaxy Note 10.1, Navigate Pain application, Aglance Solutions, Aalborg, Denmark*). Participants were asked to draw with a tablet pen (1.5 mm pen tip). Participants were asked to draw the area(s) of their pain-induced symptoms as accurately as possible and to the best of their ability. According to Graven-Nielsen¹⁴, the majority of healthy individuals express pain referral to a varying extent when similar pain models have been used. This was therefore expected to occur in this study. To ensure proper blinding and that the participants did not know that this was expected, they were specifically asked to mark all areas where they experienced pain-induced symptoms, regardless of whether these were experienced proximal or distal to the stimulation site on both sides of the body. The

subject was further instructed to paint the whole area instead of indicating it by e.g. drawing a circle around it or marking the area with a cross. Using this technique has been shown to be reliable when compared with a two dimensional paper drawing³.

For quantification of pain distribution, the body chart was divided into 3 pre-defined regions in the frontal plane (Fig. 2): The stimulation area (region 1) in and around the upper part of lower leg up to the knee, the lower part of the leg, down to a line connecting the malleoli (region 2, talocrural region), and the ankle and foot, distal to a line connecting the malleoli above (region 3, foot region). The foot region represented the previous injury area in the group with recovered pain. For each region, the pain area (expressed as number of pixels, extracted from the drawing on the tablet and reported as arbitrary units (a.u.)) was determined for individual drawings. Furthermore, the frequency of pain occurrence was expressed in each region.

Cuff algometry

A cuff algometer (*NociTech, Aalborg, Denmark and Aalborg University, Aalborg, Denmark*) was used to assess the cuff-pressure pain sensitivity, temporal summation of pain, and conditioned pain modulation^{17,41,46}. A double-chamber cuff (*VBM, Sulz, Germany*) was placed on the lower leg, with the upper rim of the cuff being in level with the tibialis anterior (covering the tibialis anterior PPT site). First, the cuff-pressure pain sensitivity was determined on the test leg and subsequently on the contralateral leg. For the cuff-pressure pain sensitivity assessments, both chambers of the cuff were inflated gradually (1 kPa/s) until the pressure became intolerable and the participant pressed a stop button upon which the cuff was immediately deflated. The participant used an electronic VAS scale to continuously rate the intensity of pressure pain where 0 cm defined 'no pain' and 10 cm anchored 'maximal pain'. Cuff-pressure pain sensitivity was investigated in three different ways: 1) The pain detection threshold (PDT) was defined as the point where the VAS score exceeded 1 cm the first time. 2) The pain tolerance threshold (PTT) was the pressure value where the subject stopped cuff inflation because of intolerable pain. Finally, 3) the pressure intensity at a VAS score of 6 cm (PVAS6) was extracted to investigate the response to a supra-pain threshold test-stimuli in accordance with previous procedures¹⁶. The PDT, PVAS6, and PTT were recorded twice for each leg and the average value used for further analysis. In case the PTT was not reached before reaching the safety limit (100kPa) of the cuff algometer, then the pain tolerance threshold was defined as 100 kPa.

Temporal summation of pain was assessed by a repetition of ten 1-s long cuff pressure stimuli with a 1-s break in between. To secure that all subjects received the same relative stimuli, the stimulation intensity was set to each individual's PTT value. When not stimulating (in the 1-s break between stimuli), a pressure of 1 kPa was kept to maintain cuff position. Reaching the designated stimulation intensity took less than 0.5 second. The subject was asked to rate the pain intensity continuously during the repeated stimulations using the electronic VAS scale although not returning towards zero between stimulations. For data analysis, the mean VAS score in the break after each stimulus was extracted. VAS data was normalized to the first stimulus and then the ratio of mean VAS score of the first four (VAS-I) and the last three (VAS-III) stimuli was calculated as the temporal summation index (TSP-ratio) as this method has previously shown good reliability in patients and healthy populations^{17,47}. An increased TSP-ratio indicates a more efficient temporal pain summation. The repeated stimulation protocol was recorded twice for each leg and the average TSP-ratio was used for further analysis.

For assessment of the conditioned pain modulation, the cuff-conditioning stimulus induced by the double cuff positioned on the leg contralateral to the test leg and inflated to 80% of PTT for that leg. Tonic pressure in the cuff was maintained while two rounds of PPT recordings were done using handheld algometry at each site on the test side. The cuff was deflated following the PPT recording. The ratio between baseline PPT values and PPT values assessed during the conditioning stimulus (where an increased CPM-ratio indicate better conditioning pain modulation) was extracted and used for analysis.

For assessment of the conditioned pain modulation, the cuff-conditioning stimulus induced by the double cuff positioned on the leg contralateral to the test leg and inflated to 80% of PTT for that leg. Tonic pressure in the cuff was maintained while two rounds of PPT recordings were done using handheld algometry at each site on the test side. The cuff was deflated following the PPT recording. The ratio between baseline PPT values and PPT values assessed during the conditioning stimulus was extracted and used for analysis.

Statistics

Parametric data are presented as mean and standard errors of the mean (SEM) and non-parametric data as median and interquartile range [IQR, 0.25 – 0.75]. For repeated measures, an adjustment for the

order effect for the injections (first vs second leg) as well as sex were performed for PPT, cuff-algometry parameters, VAS parameters, and pain referral measures.

VAS pain parameters (VAS-area, VAS-peak, and VAS duration) passed the Lilliefors test for normality and were therefore analyzed with a two-way ANOVA with *group* (recovered pain, controls) as between group factor and *leg* (test leg, contralateral leg) as repeated measure. Baseline raw PPT values were analyzed with a two-way ANOVA with *group* and *leg* factors. For each of the three assessment sites, a three-way ANOVA of the PPTs were performed with the *group* and *leg* factors as well as *time* (during, post) as repeated measures. In case of main or interaction effects, post-hoc comparisons were performed with the Newman-Keuls test adjusting for multiple comparisons. The TSP-ratio was analyzed with a Kruskal-Wallis ANOVA with comparisons both between groups and between legs. In case of main or interaction effects, post-hoc comparisons were performed with the Mann-Whitney U test with a Bonferroni correction to account for multiple comparisons. The CPM-ratio was compared between the two groups using a mixed model ANOVA with a Bonferroni correction to adjust for multiple comparisons.

The pain areas were not normal distributed and were thus analyzed with a one-way Kruskal-Wallis ANOVA across groups (all 4 legs). For post-hoc analyses, a Mann-Whitney U test was applied to detect differences between the test legs, control legs within and between groups with a Bonferroni correction applied to correct for multiple comparisons. The Bonferroni correction was applied with a factor 4 (2 groups, 2 sides). The frequency of pain reported in each region was analyzed by the Fisher's exact test. A correlation analysis was run to investigate the relationship between pain distribution and the VAS parameters. Furthermore, a correlation analysis was run to investigate the potential relationship between the duration of the last painful episode and the size of pain area in each region of the lower limb. Based on data distribution, either the Pearson's product-moment correlation or Spearman's rank correlation coefficient was calculated. For all analyses, a significance level of 0.05 was accepted.

RESULTS

Five subjects were excluded from the study; 4 persons did not feel well during saline-induced pain (two from control group) and one subject was excluded from the recovered pain group who had a previous

history of a serious disease which was not revealed at inclusion. Therefore, the data from 50 individuals (24 women), 25 in each group were included in the statistical analysis. For demographic description, see Table 1. All subjects were pain-free on the day of testing, both at rest and during the screening of active movements conducted by the primary investigator (TSP). A median duration from the painful ankle trauma of 28 days in the recovered pain group. A majority of the participants in the recovered pain group had experienced repeated episodes of pain from the ankle or foot prior to their latest one (recurring pain 19 out of 25) with full recovery between episodes. They had however, all been pain free in the two weeks prior to the experimental session.

Adjusting for sex did not change the outcomes of the below analysis and therefore the results represent both sexes. Likewise, adjusting for the sequence of testing (order effect) did not have an effect on the outcomes from the analysis.

Pressure pain sensitivity

The ANOVAs of baseline PPT at the shoulder, tibialis anterior muscle, and ankle sites, respectively, were not different between sides (test-leg, control leg) and groups (ANOVA: $F(2, 48) = 0.46$, $P < 0.5$) (Table 2). Likewise, the ANOVA of PPTs did not show effects of group during and post saline-induced pain when comparing changes from baseline in PPTs following saline injection although a tendency towards reduced PPT's was found in the previously injured leg (ANOVA: $F(2, 84) = 1.22$, $P < 0.3$) (Table 3).

Saline-induced pain intensity

The ANOVA demonstrated no significant main effects or interactions between group or legs when comparing the VAS-area (ANOVA: $F(1, 48) = 2.6$, $P < 0.1$) and VAS-peak (ANOVA: $F(1, 48) = 0.8$, $P < 0.4$), respectively, in the recovered pain group (test-leg: 1503.3 ± 157.5 cm·s; 5.7 ± 0.4 cm; contralateral leg: 1442.1 ± 142.6 cm·s; 5.7 ± 0.4 cm) and control group leg (test-leg: 1447.7 ± 159.4 cm·s; 5.8 ± 0.5 cm; contralateral leg: 1663.1 ± 159.7 cm·s; 6.1 ± 0.5 cm). Furthermore, the ANOVA showed no difference (ANOVA: $F(1, 48) = 3.3$, $P < 0.07$) when comparing the duration of pain in the control group (test leg: 980.9 ± 56.7 s; contralateral leg: 1004.8 ± 53.9 s) and the recovered pain group test (test leg: 1091.8 ± 64.8 s; contralateral leg: 1003.3 ± 43.9 s).

Saline-induced pain distribution

When comparing the groups in terms of side differences, extensive pain referral patterns were noted bilaterally in both groups (Fig. 3), with no group differences in total size between the test legs (control group: 19186 [9399 - 26249], recovered pain group: 22729 [14197 - 25945]) or the control leg (control group: 17655 [10164 - 28155], recovered pain group: 14696 [11016 - 24209], $\chi^2(3) = 1.534$; $P > 0.67$). Similarly, the total number of pain-affected regions between legs did not differ. When investigating the pain area in each region, the Kruskal-Wallis ANOVA demonstrated a significant effect for the foot region ($\chi^2(3) = 9.235$, $P < 0.002$), with larger pain areas in the test leg in the recovered pain group compared with the test leg in the control group (Chi-square $P < 0.01$; Fig. 3; Table 4). Furthermore, hypertonic saline evoked referred pain more frequently in the foot region in the test leg of the recovered pain group when compared with the control group (Fisher's exact test: $P < 0.005$, Table 5).

No correlation was found between the size of the pain referral pattern and pain intensity following hypertonic saline injection (Spearman's rho: $P > 0.05$). Likewise, no correlation was found between the duration of pain (in days) and the size of referred pain in any of the regions in the lower limb (Spearman's rho: $P > 0.05$).

Tonic pressure-induced pain distribution

No group or leg differences were found when comparing the total pain distribution following tonic pressure pain stimulation at baseline or post saline-induced pain (Table 6). However, the Fisher's exact test demonstrated that tonic pressure stimulation at baseline evoked referred pain in the talocrural region of the test leg more frequently in the recovered pain group compared with the control group (Fisher's exact test: $P < 0.01$, Table 5).

Cuff pain detection threshold and pain tolerance threshold

For the pain tolerance threshold, 5 subjects in the recovered pain group and three subjects in the control group reached the machine's cut-off (100 kPa). This value was therefore registered as their pain tolerance threshold. No difference was found when comparing the PDT or PTT between the legs and groups (Table 5). However, the ANOVA of the PVAS6 demonstrated an interaction between group and leg (ANOVA: $F(1, 48) = 4.09$, $P < 0.049$) with a post-hoc analysis revealing higher PVAS6 in the test leg compared with the control leg for the recovered pain group (Table 5; NK: $P < 0.03$).

Temporal pain summation and conditioning pain modulation

The tests of temporal summation, revealed no differences in the ratio between VAS-I and VAS-III between the recovered pain group (test leg: median 1.8 [1.2 – 2.8], control leg: 1.4 [1.1 – 1.9]) and the control group (test leg: median 1.2 [1.0 – 1.7], control leg: 1.3 [1.0 – 2.0], Kruskal-Wallis: $\chi^2(3) = 4.524$, $P < 0.2$). For the CPM effect, the ANOVA demonstrated a significant interaction between 'group' and 'sites' (ANOVA: $F(2, 96) = 4.6$, $P < 0.01$) where the CPM effect at the ankle was greater in the recovered pain group ($26.9 \pm 4.9\%$) compared with the control group ($8.5 \pm 5.5\%$, $P < 0.016$). No difference in CPM response was found when comparing deltoid (recovered pain group: $15.5 \pm 4.3\%$, control group: $21.2\% \pm 5.4\%$) or the injection site (recovered pain group: $38.8 \pm 7.8\%$, control group: $20.9 \pm 5.8\%$).

DISCUSSION

This explorative study is the first to assess pain characteristics in individuals recently recovered from ankle pain. Although baseline characteristics were comparable, conditioned pain modulation was seen to be improved around the ankle in the recovered pain group. Moreover, the extent and distribution of the referred pain pattern in response to experimental pain manifested as an enlarged pain referral area, specifically in the ankle region in the recovered pain group. Together, these findings suggest a selective rather than generalized spread in nociceptive processing when recovered from ankle pain.

Increased pain referral and the sensitized pain system

Referred pain is defined as pain felt remote to the site of origin/stimulation¹. It is well known in clinical practice^{2,5,9,26,35} and reproducible in experimental settings^{34,37}.

The hypertonic saline model caused a more extensive pain referral than tonic pressure, similar to previous findings⁸ and may relate to differences in pain intensity. Painful pressure results in a time-related increase in pain area¹³ which may indicate that the participants in this study were under-stimulated by only receiving a 60s stimulation with PPT+20%. However, pain intensity during tonic pressure was not registered in this study, making comparisons to previous findings speculative.

The injection site in this study receives innervation from spinal segments L4-S3⁴² and the talocrural joint (which is commonly affected in ankle injuries) receives innervation from mid and

lower levels of the lumbosacral plexus⁴². Therefore, a neuroanatomical overlap exists between the spinal segments receiving afferent information from both sites. This overlap may explain why healthy individuals in the current and previous studies^{12,13}, show distal expansions of referred pain following hypertonic saline.

Despite the group differences in pain referral, it is noteworthy that the median size of pain area over the ankle and foot was relatively small in the control group compared with the recovered pain group (Table 6). Considering the random order of saline injections into the test leg and control leg in both groups, this may be difficult to explain. The differences may however be driven by the smaller number of controls reporting pain in the area (N=16) compared with the recovered pain group (N=24, Table 4) in addition to the large variability in referred pain within the groups (table 6).

Injury-induced changes in nociceptive processing

No significant differences were found for measures of pressure pain sensitivity and temporal pain summation. Considering that widespread pain is a common feature in clinical populations^{22,44}, the current findings suggest that the two groups are responding in a similar, normal manner.

Interestingly, higher cuff-pressure was needed to evoke pain equal to 6 cm on VAS in the previously painful leg, compared to the contralateral leg in the recovered pain group. Furthermore, a higher CPM response was seen at the talocrural joint in the recovered pain group compared with controls. This may reflect an ongoing, selective regional gain of pain inhibition, and may potentially be considered a healthy, adaptive response. The authors are unaware of studies demonstrating such a selective shift in pain inhibition following recovery from a painful injury. If this occurs, the current findings may reflect a protective mechanism that might be part of the recovery process. A prospective cohort study is needed to investigate if this occurs when recovering from an injury. The seemingly increased efficiency of descending inhibitory mechanisms contrasts the facilitated referred pain to the previously painful ankle seen here. However, recent findings indicate that a painful conditioning stimulus does not affect the size of pain referral evoked by experimental pain²⁸, suggesting that mechanisms for referred pain and descending pain control appear to be active, independent of each other.

This present study observed that following full recovery from a recent period of ankle pain, resulted in an increase in area and frequency of experimentally-induced referred pain over the ankle region. Although the actual area of expansion is relatively small, this may relate to the ankle region being small compared to the lower leg as a whole. These findings are in line with previous observations where a painful experimental stimulus resulted in a pain projection towards the a site that was previously the locus of a strong nociceptive stimulus²¹.

Approximately 80% of healthy subjects develop pain in the ankle area following hypertonic saline injections into the tibialis anterior muscle¹⁴ which is consistent with the frequency of referred pain in the control group in the present study. Interestingly, this ratio is shifted in the recovered pain group where almost all subjects reported pain in the ankle region (23/25) and the foot region (24/25). Existing evidence suggests that this may occur via expanding receptive fields at the spinal level²⁰ and sensitization of central rather than peripheral mechanisms^{32,48}. Moreover, recent experimental findings^{30,36} show that processing of pain intensity and spatial distribution of symptoms does not involve the same brain areas. In his study, subjects performed discriminative tasks consisting of rating pain intensity followed by recording pain location. Supraspinal activity during spatial focus may in this way shift information processing away from the intensity of the nociceptive stimulus³⁰. The intensity and duration of the hypertonic saline-induced pain profile were similar to what has been shown previously¹³ with no difference found between the two groups. Further, no relationship was found between spatial distribution of pain and VAS parameters in contrast to previous findings^{14,38}. Although speculative, this may explain the group differences in pain distribution despite similarities in VAS profiles seen in the current study. It is known that long-term changes in synaptic strengthening and central nervous system re-organization are related to the intensity and duration of the initial painful stimulus²³ but even low intensity, on-going nociceptive stimuli can maintain sensitization of central pain mechanisms⁴⁹. This may imply that the relatively long duration of the last painful clinical episode (Table 1) may have contributed to functional changes in the central nervous system resulting in the shifted pain referral pattern.

Limitations and perspectives

The control group consisted of individuals with no current or previous history of ongoing pain. However, even though it is questionable whether individuals over 18 years may have gone through life without sustaining an injury, this would have provided a more conservative estimate of the group differences in the present study.

Two main limitations in this study are the divisions used to allocate the ankle regions and subsequent areas of pain and the time of the initial ankle injury to study onset were not uniform (Table 1). The divisions used in this study reflect anatomical landmarks and were based on a-priori knowledge of pain referral patterns^{12,13}. The present findings were a result of an exploratory investigation and require a prospective follow-up to determine whether referred pain can be increased by residual neuroplasticity expressed as e.g. enhanced functional connectivity of synaptic connections in the central pain system^{14,25,40}. The transition from injury to recovery as assessed by on-going pain or functional measures could be standardized by systematically assessing individuals at regular intervals following injury prospectively. This might clarify whether extended pain referral patterns remain or dissipate over. Another consideration is that a significant proportion of the asymptomatic individuals experienced repeated episodes of ankle pain and thus may represent a subgroup e.g. recurrent versus initial ankle injuries. This study was however, underpowered to deal with such an investigation. Given the explorative nature of this study, these findings can be fruit for further investigations.

In general, the statistical findings favor the research hypothesis. However, the large variation in pain area suggests that injuries and/or referral patterns are not uniform. Therefore, it must be acknowledged that the diagnostic utility of these findings is limited. Furthermore, some of the findings seem not only driven by extended pain referral in the recovered pain group, but also by a smaller proportion of controls reporting pain at the most distal sites (see e.g. table 4). Lastly, it is important to consider that multiple comparisons within and between groups were made in this study. Even though these were controlled for, it is plausible that some of the findings are random.

Although the size of pain area is not related with catastrophizing thoughts in patients with chronic musculoskeletal pain²⁹, it seems to be different in more acute pain conditions¹⁹. An assessment of catastrophizing thoughts is therefore warranted in future studies. Despite limitations, the findings raise queries as to whether individuals with a recent history of pain are subject to a continuous and peripheral nociceptive input that is suppressed by an increased gain in pain inhibition. Residual effects

in the spinal or supraspinal system would account for the dynamic nature of recurrent or episodic pain and indicate the involvement of central pain mechanisms.

Conclusion

This exploratory study shows an expansion in referred pain patterns to a previous pain area in otherwise asymptomatic individuals. In the recovered pain participants, an increased gain in the descending inhibitory systems was also found. These preliminary findings warrant a larger prospective study to clarify the time profile and functional relevance for e.g. recurrent pain episodes.

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Table 1. Demographic data (N=50, 25 in each group) is presented as mean (range), sex distribution as percentage of females in each group. Time in days since subjects last had pain from the ankle are presented as median [IQR]. Number of days away from full physical activity following injury are presented as median [IQR]. The number of subjects with a recurring pain problem related to the ankle or foot is presented.

	Sex (women)	Age (years)	Height (cm)	Weight (kg)	Post-pain time (days)	Duration of pain-induced disability (days)	Recurring pain episodes (% subjects)
Control group	44% (N=11)	25 (19 - 35)	176 (156 - 194)	74 (51 - 110)	N/A	N/A	N/A
Recovered pain group	52% (N=13)	24 (18 - 30)	178 (157 - 200)	75 (49 - 102)	28 [14 - 56]	19 [7 - 42]	76% (N=19)

Table 2. Mean (\pm SEM, N=25) baseline pressure pain thresholds (PPT) at the three assessment sites. No significant group differences were found ($P > 0.05$). Note: The test leg in recovered pain group reflects ankle with prior injury or pain.

Baseline				
Pressure pain thresholds (kPa)		Tibialis anterior	Medial deltoid	Talocrural joint
Control group	Test leg	431.6 \pm 36.6	342.0 \pm 27.5	397.6 \pm 21.4
	Control leg	430.3 \pm 35.0	350.0 \pm 31.1	412.9 \pm 23.6
Recovered pain Group	Test leg	390.6 \pm 31.9	329.1 \pm 30.1	401.1 \pm 27.9
	Control leg	402.6 \pm 30.1	335.7 \pm 31.0	408.5 \pm 24.7

Table 3. Mean (\pm SEM, N=25) change in pressure pain thresholds (% of baseline) at the three assessment sites during saline-induced pain and post saline-induced pain on the test leg and the contralateral leg, respectively. Note that the percentage values depict changes that occurred when injection was given on the same side. No significant group differences were found ($P > 0.05$). Note: The test leg in the recovered pain group reflects ankle with prior injury or pain.

During saline-induced pain				
Pressure pain thresholds (kPa) (% of baseline)		Tibialis anterior	Medial deltoid	Talocrural joint
Control group	Test leg	97.2 \pm 7.9	117.9 \pm 4.6	105.1 \pm 5.3
	Contralateral leg	86.4 \pm 8.0	122.7 \pm 6.0	104.7 \pm 6.7
Recovered pain group	Test leg	83.7 \pm 6.2	112.8 \pm 4.8	103.7 \pm 4.7
	Contralateral leg	83.1 \pm 4.9	115.7 \pm 5.3	95.9 \pm 5.7
Post saline-induced pain				
Control group	Test leg	99.9 \pm 6.7	100.9 \pm 4.7	109.6 \pm 4.6
	Contralateral leg	93.8 \pm 7.7	104.8 \pm 4.0	109.7 \pm 3.9
Recovered pain group	Test leg	98.5 \pm 7.1	94.7 \pm 3.9	107.9 \pm 4.2
	Contralateral leg	89.7 \pm 5.6	98.6 \pm 5.4	102.5 \pm 3.8

Table 4 Frequency of affected areas after a painful stimulus (Injection area (area 1) = around the injection site, Distal lower leg (area 2) = lowest 1/3 of leg and Ankle and foot (area 3) = foot and ankle). Data are presented as sum of affected areas in the test leg and contralateral leg for each group (N=25). Significant difference compared with the same side leg in the control group (Fisher's exact: *, $P < 0.05$)

			Regions on the lower limb		
			Injection area	Distal lower leg	Ankle and foot
Tonic pressure baseline (pre-saline)	Control group	Test leg	23	0	2
		Contralateral leg	23	3	3
	Recovered pain group	Test leg	24	6*	5
		Contralateral leg	24	5	7
Hypertonic saline	Control group	Test leg	23	20	16
		Contralateral leg	23	21	20
	Recovered pain group	Test leg	24	23	24*
		Contralateral leg	24	22	22
Tonic pressure post-saline	Control group	Test leg	23	7	7
		Contralateral leg	22	6	6
	Recovered pain group	Test leg	24	6	9

	Contralateral leg	22	7	11
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Table 5 Mean (\pm SEM) cuff pressure needed to reach the pain detection threshold (PDT), to reach VAS 6 cm (PVAS6), and pain tolerance threshold (PTT). Data are presented for the test leg and the contralateral leg. No significant group or leg differences were found for PDT and PTT (ANOVA: $P > 0.05$), but a significant difference in PVAS6 was found between the test leg and contralateral leg in the recovered pain group (ANOVA: *, $P < 0.05$).

Cuff algometry				
		Control group	Recovered pain group	
PDT (kPa)	Test leg	25.7 \pm 2.4	27.7 \pm 1.9	
	Contralateral leg	27.1 \pm 2.5	26.4 \pm 1.9	
PVAS6 (kPa)	Test leg	44.6 \pm 3.3	49.5 \pm 3.4*	
	Contralateral leg	44.6 \pm 3.0	43.8 \pm 2.9	
PTT (kPa)	Test leg	66.1 \pm 4.2	70.6 \pm 4.6	
	Contralateral leg	64.0 \pm 3.8	64.5 \pm 4.8	

Table 6 Median [*IQR*] Size of pain areas indicated in arbitrary units following suprathreshold stimulation at baseline (left column), hypertonic saline (middle column) and following suprathreshold stimulation post-pain (right column). Significant difference compared with the same side leg in the control group (Kruskal-Wallis: *, $P < 0.05$).

			Pressure (pre-saline)	Hypertonic saline	Pressure (post-saline)
Region 1 (stimulation area)	Control group	Test leg	2168 [789 - 2686]	2195 [251 - 5608]	2047 [907 - 4612]
		Contralateral leg	1509 [225 - 4075]	3087 [1425 - 4127]	2281 [1515 - 4468]
	pain group	Test leg	1495 [375 - 3348]	2387 [1150 - 4028]	2632 [1873 - 4433]
		Contralateral leg	1446 [909 - 2943]	3014 [1412 - 4269]	2581 [1297 - 3531]
Region 2 (lower leg)	Control group	Test leg	0 [0 - 0]	4451 [518 - 5401]	0 [0 - 0]
		Contralateral leg	0 [0 - 0]	4054 [1191 - 4922]	0 [0 - 796]
	Recovered pain group	Test leg	0 [0 - 0]	2872 [1359 - 4556]	0 [0 - 434]
		Contralateral leg	0 [0 - 0]	2216 [1165 - 3624]	0 [0 - 334]
Region 3 (ankle and foot)	Control group	Test leg	0 [0 - 0]	169 [0 - 4071]	0 [0 - 0]
		Contralateral leg	0 [0 - 0]	1765 [443 - 5396]	0 [0 - 428]
	Recovered pain group	Test leg	0 [0 - 0]	3420 [1369 - 6707]*	0 [0 - 901]
		Contralateral leg	0 [0 - 1760]	2822 [1233 - 5246]	0 [0 - 2510]

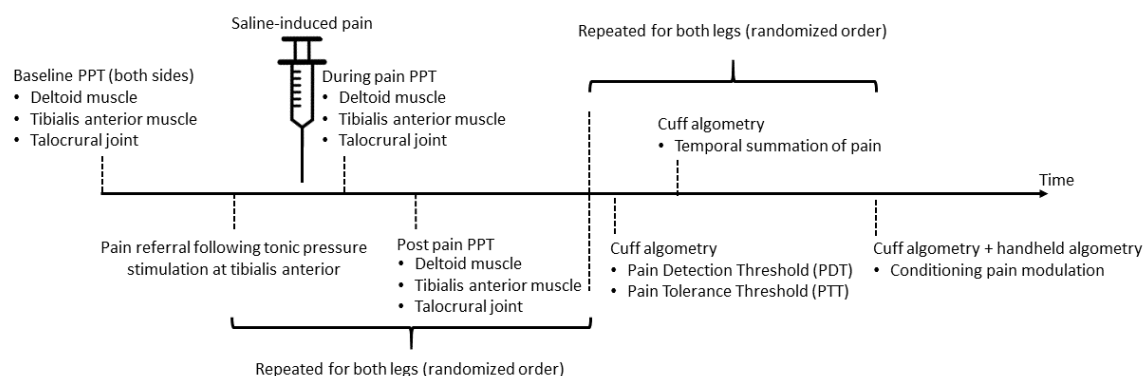


Figure 1. The chronological order of the testing sequence. Measurements of pressure pain sensitivity and pain referral (mechanical and saline-induced) were performed in a randomized (leg) balanced order. When both legs had been tested, the same sequence was used to assess PDT and PTT using cuff algometry. The PTT value was used to determine the stimulation intensity for temporal summation of pain and the painful conditioning stimulus used for assessing conditioning pain modulation. The experimental session lasted 90 minutes in both groups.

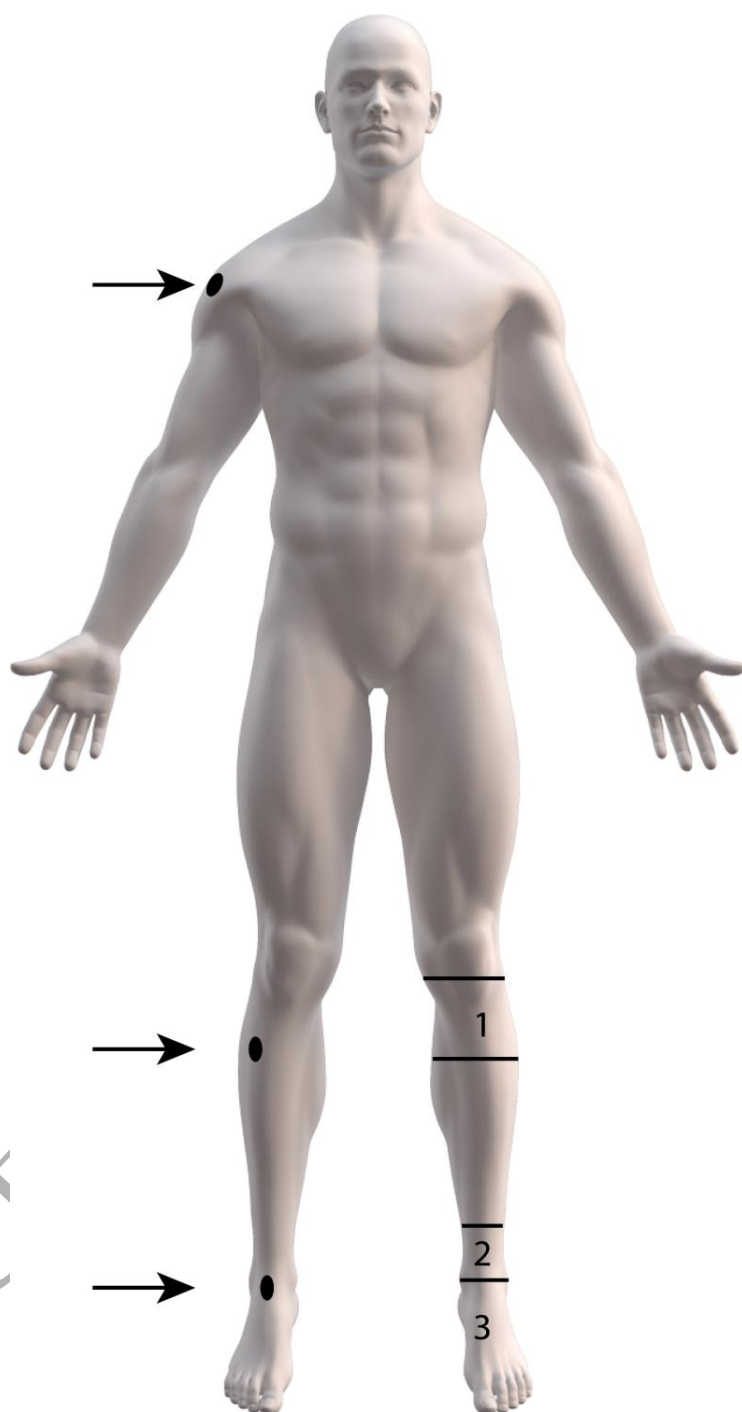


Figure 2. Location of assessment sites (indicated with arrows) for pressure algometry (left), and outlines of body regions (1, 2 and 3) used for quantification of pain distribution following experimental pain (right). All assessments were performed bilaterally although illustrated unilaterally.

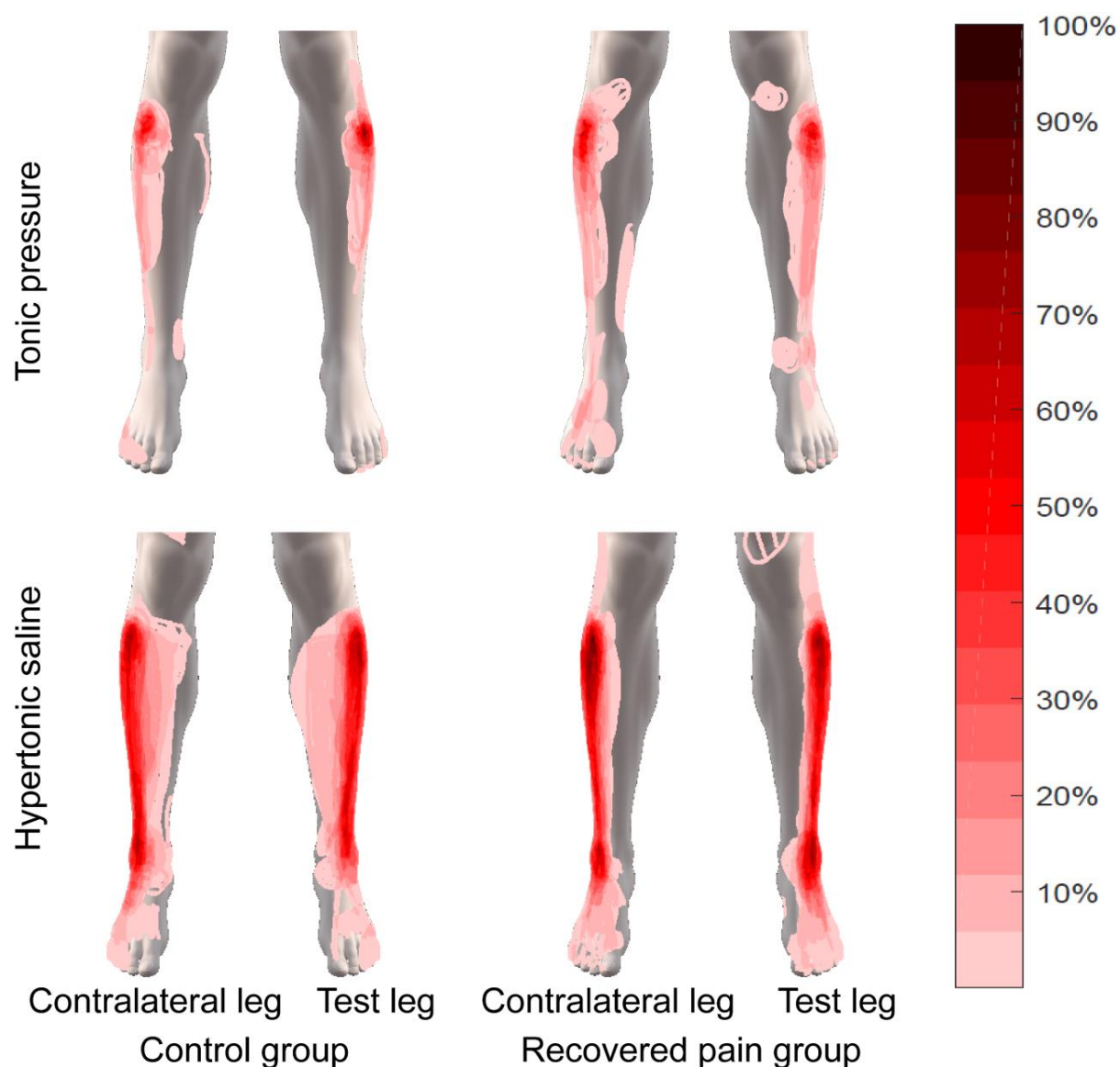


Figure 3. The area and location of pain referral following suprathreshold stimulation at baseline (above) and hypertonic saline injection (below) in the control group, and the recovered pain group. Drawings are presented as overlays of all the original individual pain drawings for group on the test leg (previously injured leg in the recovered pain group and dominating leg in controls) and the contralateral leg (control leg). For the tonic pressure showed dominant areas of pain reports at the site of the stimulation and for the recovered pain group the pain reports also occurred more distally. In contrast, hypertonic saline showed in general showed a greater area of referred pain with the recovered

pain group reporting pain more often over the ankle area. The color-coding (shading) shows the percentage of individuals within each group (N=25) reporting pain below the knee and ankle regions. Note the image is best viewed in color or greyscale.

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